

SEQUENCE PROTOCOL

<110> Degussa-Hüls AG
Forschungszentrum-Jülich GmbH

<120> New nucleotide sequences coding for the thrE gene and process
for the enzymatic production of L-threonine with coryneform
bacteria.

<130> 990079 BT

<140>

<141>

<160> 4

<170> Patent Proprietor Publication 2.1

<210> 1

<211> 2817

<212> DNA

<213> Corynebacterium glutamicum ATCC14752

<220>

<221> CDS

<222> (398)..(1864)

<223> thrE-Gen

<400> 1

(A list of gene sequences is given at Line 30, German page 23 to Line
36, German page 31.)

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agccaaggga aaagaaagcc cctaagcccc gtgttattaa atggagactc tttggagacc 120

tcaagccaaa aaggggcatt ttcattaaga aaatacccct ttgacctggt gttattgagc 180

tggagaagag acttgaactc tcaacctacg cattacaagt gcgttgcgct gccaatgacg 240

ccactccagc accgcagatg ctgatgatca acaactacga atacgtatct tagcgtatgt 300

gtacatcaca atggaattcg gggctagagt atctggtgaa ccgtgcataa acgacctgtg 360

attggactct ttttccttgc aaaatgtttt ccagcgg atg ttg agt ttt gcg acc 415
Met Leu Ser Phe Ala Thr
1 5

ctt cgt ggc cgc att tca aca gtt gac gct gca aaa gcc gca cct ccg 463
Leu Arg Gly Arg Ile Ser Thr Val Asp Ala Ala Lys Ala Ala Pro Pro
10 15 20

cca tcg cca cta gcc ccg att gat ctc act gac cat agt caa gtg gcc 511
Pro Ser Pro Leu Ala Pro Ile Asp Leu Thr Asp His Ser Gln Val Ala
25 30 35

ggt gtg atg aat ttg gct gcg aga att ggc gat att ttg ctt tct tca 559
Gly Val Met Asn Leu Ala Ala Arg Ile Gly Asp Ile Leu Leu Ser Ser
40 45 50

ggt acg tca aac agt gat acc aag gtg caa gtt cga gog gtg acc tct 607
Gly Thr Ser Asn Ser Asp Thr Lys Val Gln Val Arg Ala Val Thr Ser
55 60 65 70

65

0996551.09201

506 BT

gcg tat ggc ctg tac tat acg cat gtg gat atc acg ttg aat acg atc 655
Ala Tyr Gly Leu Tyr Tyr Thr His Val Asp Ile Thr Leu Asn Thr Ile
75 80 85

5 acc atc ttc acc aac atc ggt gtg gag agg aag atg ccg gtc aac gtg 703
Thr Ile Phe Thr Asn Ile Gly Val Glu Arg Lys Met Pro Val Asn Val
90 95 100

10 ttt cat gtt gtg ggc aag ttg gac acc aac ttc tcc aaa ctg tct gag 751
Phe His Val Val Gly Lys Leu Asp Thr Asn Phe Ser Lys Leu Ser Glu
105 110 115

15 gtt gac cgt ttg atc cgt tcc att cag gct ggt gct acc ccg cct gag 799
Val Asp Arg Leu Ile Arg Ser Ile Gln Ala Gly Ala Thr Pro Pro Glu
120 125 130

20 gtt gcc gag aaa att ctg gac gag ttg gag caa tcg cct gcg tct tat 847
Val Ala Glu Lys Ile Leu Asp Glu Leu Glu Gln Ser Pro Ala Ser Tyr
135 140 145 150

25 ggt ttc cct gtt gcg ttg ctt ggc tgg gca atg atg ggt ggc gct gtt 895
Gly Phe Pro Val Ala Leu Leu Gly Trp Ala Met Met Gly Gly Ala Val
155 160 165

30 gct gtg ctg ttg ggt ggt gga tgg cag gtt tcc cta att gct ttt att 943
Ala Val Leu Leu Gly Gly Gly Trp Gln Val Ser Leu Ile Ala Phe Ile
170 175 180

35 acc gcg ttc acg atc att gcc acg acg tca ttt ttg gga aag aag ggt 991
Thr Ala Phe Thr Ile Ile Ala Thr Thr Ser Phe Leu Gly Lys Lys Gly
185 190 195

40 ttg cct act ttc ttc caa aat gtt gtt ggt ggt ttt att gcc acg ctg 1039
Leu Pro Thr Phe Phe Gln Asn Val Val Gly Gly Phe Ile Ala Thr Leu
200 205 210

45 cct gca tcg att gct tat tct ttg gcg ttg caa ttt ggt ctt gag atc 1087
Pro Ala Ser Ile Ala Tyr Ser Leu Ala Leu Gln Phe Gly Leu Glu Ile
215 220 225 230

50 aaa ccg agc cag atc atc gca tct gga att gtt gtg ctg ttg gca ggt 1135
Lys Pro Ser Gln Ile Ile Ala Ser Gly Ile Val Val Leu Leu Ala Gly
235 240 245

55 ttg aca ctt gtg caa tct ctg cag gac ggc atc acg ggc gct ccg gtg 1183
Leu Thr Leu Val Gln Ser Leu Gln Asp Gly Ile Thr Gly Ala Pro Val
250 255 260

60 aca gca agt gca cga ttt ttt gaa aca ctc ctg ttt acc ggc ggc att 1231
Thr Ala Ser Ala Arg Phe Phe Glu Thr Leu Leu Phe Thr Gly Gly Ile
265 270 275

65 gtt gct ggc gtg ggt ttg ggc att cag ctt tct gaa atc ttg cat gtc 1279
Val Ala Gly Val Gly Leu Gly Ile Gln Leu Ser Glu Ile Leu His Val
280 285 290

atg ttg cct gcc atg gag tcc gct gca gca cct aat tat tcg tct aca 1327
Met Leu Pro Ala Met Glu Ser Ala Ala Ala Pro Asn Tyr Ser Ser Thr
295 300 305 310

65 ttc gcc cgc att atc gct ggt ggc gtc acc gca gcg gcc ttc gca gtg 1375
Phe Ala Arg Ile Ile Ala Gly Gly Val Thr Ala Ala Ala Phe Ala Val
315 320 325

09963551 092701

ggt tgt tac gcg gag tgg tcc tcg gtg att att gcg ggg ctt act gcg 1423
 Gly Cys Tyr Ala Glu Trp Ser Ser Val Ile Ile Ala Gly Leu Thr Ala
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5 ctg atg ggt tct gcg ttt tat tac ctc ttc gtt gtt tat tta ggc ccc 1471
 Leu Met Gly Ser Ala Phe Tyr Tyr Leu Phe Val Val Tyr Leu Gly Pro
 345 350 355

10 gtc tct gcc gct gcg att gct gca aca gca gtt ggt ttc act ggt ggt 1519
 Val Ser Ala Ala Ala Ile Ala Ala Thr Ala Val Gly Phe Thr Gly Gly
 360 365 370

15 ttg ctt gcc cgt cga ttc ttg att cca ccg ttg att gtg gcg att gcc 1567
 Leu Leu Ala Arg Arg Phe Leu Ile Pro Pro Leu Ile Val Ala Ile Ala
 375 380 385 390

20 ggc atc aca cca atg ctt cca ggt cta gca att tac cgc gga atg tac 1615
 Gly Ile Thr Pro Met Leu Pro Gly Leu Ala Ile Tyr Arg Gly Met Tyr
 395 400 405

gcc acc ttg aat gat caa aca ctc atg ggt ttc acc aac att gcg gtt 1663
 Ala Thr Leu Asn Asp Gln Thr Leu Met Gly Phe Thr Asn Ile Ala Val
 410 415 420

25 gct tta gcc act gct tca tca ctt gcc gct ggc gtg gtt ttg ggt gag 1711
 Ala Leu Ala Thr Ala Ser Ser Leu Ala Ala Gly Val Val Leu Gly Glu
 425 430 435

30 tgg att gcc cgc agg cta cgt cgt cca cca cgc ttc aac cca tac cgt 1759
 Trp Ile Ala Arg Arg Leu Arg Arg Pro Pro Arg Phe Asn Pro Tyr Arg
 440 445 450

35 gca ttt acc aag gcg aat gag ttc tcc ttc cag gag gaa gct gag cag 1807
 Ala Phe Thr Lys Ala Asn Glu Phe Ser Phe Gln Glu Glu Ala Glu Gln
 455 460 465 470

aat cag cgc cgg cag aga aaa cgt cca aag act aat caa aga ttc ggt 1855
 Asn Gln Arg Arg Gln Arg Lys Arg Pro Lys Thr Asn Gln Arg Phe Gly
 475 480 485

40 aat aaa agg taaaaatcaa cctgcttagg cgtctttcgc ttaaatagcg 1904
 Asn Lys Arg

45 tagaatatcg ggtcgatcgc ttttaaacac tcaggaggat ccttgccggc caaaatcacg 1964
 gacactcgtc ccaccccgaga atcccttcac gctgttgaag aggaaaccgc agccggtgcc 2024
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<212> PRT

15 <213> Corynebacterium glutamicum ATCC14752

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 25 Asp His Ser Gln Val Ala Gly Val Met Asn Leu Ala Ala Arg Ile Gly
 35 40 45
 Asp Ile Leu Leu Ser Ser Gly Thr Ser Asn Ser Asp Thr Lys Val Gln
 50 55 60
 30 Val Arg Ala Val Thr Ser Ala Tyr Gly Leu Tyr Tyr Thr His Val Asp
 65 70 75 80
 Ile Thr Leu Asn Thr Ile Thr Ile Phe Thr Asn Ile Gly Val Glu Arg
 85 90 95
 35 Lys Met Pro Val Asn Val Phe His Val Val Gly Lys Leu Asp Thr Asn
 100 105 110
 Phe Ser Lys Leu Ser Glu Val Asp Arg Leu Ile Arg Ser Ile Gln Ala
 115 120 125
 Gly Ala Thr Pro Pro Glu Val Ala Glu Lys Ile Leu Asp Glu Leu Glu
 130 135 140
 45 Gln Ser Pro Ala Ser Tyr Gly Phe Pro Val Ala Leu Leu Gly Trp Ala
 145 150 155 160
 Met Met Gly Gly Ala Val Ala Val Leu Leu Gly Gly Gly Trp Gln Val
 165 170 175
 50 Ser Leu Ile Ala Phe Ile Thr Ala Phe Thr Ile Ile Ala Thr Thr Ser
 180 185 190
 Phe Leu Gly Lys Lys Gly Leu Pro Thr Phe Phe Gln Asn Val Val Gly
 195 200 205
 Gly Phe Ile Ala Thr Leu Pro Ala Ser Ile Ala Tyr Ser Leu Ala Leu
 210 215 220
 60 Gln Phe Gly Leu Glu Ile Lys Pro Ser Gln Ile Ile Ala Ser Gly Ile
 225 230 235 240
 Val Val Leu Leu Ala Gly Leu Thr Leu Val Gln Ser Leu Gln Asp Gly
 245 250 255
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Ile Thr Gly Ala Pro Val Thr Ala Ser Ala Arg Phe Phe Glu Thr Leu
 260 265 270
 5 Leu Phe Thr Gly Gly Ile Val Ala Gly Val Gly Leu Gly Ile Gln Leu
 275 280 285
 Ser Glu Ile Leu His Val Met Leu Pro Ala Met Glu Ser Ala Ala Ala
 290 295 300
 10 Pro Asn Tyr Ser Ser Thr Phe Ala Arg Ile Ile Ala Gly Gly Val Thr
 305 310 315 320
 Ala Ala Ala Phe Ala Val Gly Cys Tyr Ala Glu Trp Ser Ser Val Ile
 325 330 335
 15 Ile Ala Gly Leu Thr Ala Leu Met Gly Ser Ala Phe Tyr Tyr Leu Phe
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 20 Val Val Tyr Leu Gly Pro Val Ser Ala Ala Ala Ile Ala Ala Thr Ala
 355 360 365
 Val Gly Phe Thr Gly Gly Leu Leu Ala Arg Arg Phe Leu Ile Pro Pro
 370 375 380
 25 Leu Ile Val Ala Ile Ala Gly Ile Thr Pro Met Leu Pro Gly Leu Ala
 385 390 395 400
 Ile Tyr Arg Gly Met Tyr Ala Thr Leu Asn Asp Gln Thr Leu Met Gly
 405 410 415
 30 Phe Thr Asn Ile Ala Val Ala Leu Ala Thr Ala Ser Ser Leu Ala Ala
 420 425 430
 Gly Val Val Leu Gly Glu Trp Ile Ala Arg Arg Leu Arg Arg Pro Pro
 435 440 445
 Arg Phe Asn Pro Tyr Arg Ala Phe Thr Lys Ala Asn Glu Phe Ser Phe
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 cgccactcca gcaccgcaga tgctgatgat caacaactac gaatacgtat cttagcgtat 180
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5	acc ctt cgt ggc cgc att tca aca gtt gac gct gca aaa gcc gca cct Thr Leu Arg Gly Arg Ile Ser Thr Val Asp Ala Ala Lys Ala Pro 10 15 20	342				
10	ccg cca tcg cca cta gcc ccg att gat ctg act gac cat agt caa gtg Pro Pro Ser Pro Leu Ala Pro Ile Asp Leu Thr Asp His Ser Gln Val 25 30 35	390				
15	gcc ggt gtg atg aat ttg gct gcg aga att ggk gat att ttg ctt tct Ala Gly Val Met Asn Leu Ala Ala Arg Ile Gly Asp Ile Leu Leu Ser 40 45 50	438				
20	tca ggt acg tca aat agt gac acc aag gta caa gtt cga gca gtg acc Ser Gly Thr Ser Asn Ser Asp Thr Lys Val Gln Val Arg Ala Val Thr 55 60 65	486				
25	tct gcg tac ggt ttg tac tac acg cac gtg gat atc acg ttg aat acg Ser Ala Tyr Gly Leu Tyr Tyr Thr His Val Asp Ile Thr Leu Asn Thr 70 75 80 85	534				
30	atc acc atc ttc acc aac atc ggt gtg gag agg aag atg ccg gtc aac Ile Thr Ile Phe Thr Asn Ile Gly Val Glu Arg Lys Met Pro Val Asn 90 95 100	582				
35	gtg ttt cat gtt gta gcc aag ttg gac acc aac ttc tcc aaa ctg tct Val Phe His Val Val Gly Lys Leu Asp Thr Asn Phe Ser Lys Leu Ser 105 110 115	630				
40	gag gtt gac cgt ttg atc cgt tcc att cag gct ggt gcg acc ccg cct Glu Val Arg Leu Ile Arg Ser Ile Gln Ala Gly Ala Thr Pro Pro 120 125 130	678				
45	gat gtt gcc gag aaa atc ctg gac gag ttg gag caa tcc cct gcg tct Leu Val Ala Glu Lys Ile Leu Asp Glu Leu Glu Gln Ser Pro Ala Ser 135 140 145	726				
50	tat ggt ttc cct gtt gcg ttg ctt ggc tgg gca atg atg ggt ggt gct Tyr Gly Phe Pro Val Ala Leu Leu Gly Trp Ala Met Met Gly Gly Ala 150 155 160 165	774				
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65	ggk ttg cct act ttc ttc caa aat gtt gtt ggt ggt ttt att gcc acg Gly Leu Pro Thr Phe Phe Gln Asn Val Val Gly Gly Phe Ile Ala Thr 200 205 210	918				
70	ctg cct gca tcg att gct tat tct ttg gcg ttg caa ttt ggt ctt gag Leu Pro Ala Ser Ile Ala Tyr Ser Leu Ala Leu Gln Phe Gly Leu Glu 215 220 225	966				
75	atc aaa ccg agc cag atc atc gca tct gga att gtt gtg ctg ttg gca Ile Lys Pro Ser Gln Ile Ile Ala Ser Gly Ile Val Val Leu Leu Ala 230 235 240 245	1014				

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 250 255 260

5 gtg aca gca agt gca cga ttt ttc gaa aca ctc ctg ttt acc ggc ggc 1110
 Val Thr Ala Ser Ala Arg Phe Phe Glu Thr Leu Leu Phe Thr Gly Gly
 265 270 275

10 att gtt gct ggc gtg ggt ttg ggc att cag ctt tct gaa atc ttg cat 1158
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 295 300 305

20 aca ttc gcc cgc att atc gct ggt ggc gtc acc gca gcg gcc ttc gca 1254
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 310 315 320 325

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 Val Gly Cys Tyr Ala Glu Trp Ser Ser Val Ile Ile Ala Gly Leu Thr
 330 335 340

25 gcg ctg atg ggt tct gcg ttt tat tac ctc ttc gtt gtt tat tta ggc 1350
 Ala Leu Met Gly Ser Ala Phe Tyr Tyr Leu Phe Val Val Tyr Leu Gly
 345 350 355

30 ccc gtc tct gcc gct gcg att gct gca aca gca gtt ggt ttc act ggt 1398
 Pro Val Ser Ala Ala Ala Ile Ala Ala Thr Ala Val Gly Phe Thr Gly
 360 365 370

35 ggt ttg ctt gcc cgt cga ttc ttg att cca ccg ttg att gtg gcg att 1446
 Gly Leu Leu Ala Arg Arg Phe Leu Ile Pro Pro Leu Ile Val Ala Ile
 375 380 385

40 gcc ggc atc aca cca atg ctt cca ggt cta gca att tac gcg gga atg 1494
 Ala Gly Ile Thr Pro Met Leu Pro Gly Leu Ala Ile Tyr Arg Gly Met
 390 395 400 405

tac gcc acc ctg aat gat caa aca ctc atg ggt ttc acc aac att gcg 1542
 Tyr Ala Thr Leu Asn Asp Gln Thr Leu Met Gly Phe Thr Asn Ile Ala
 410 415 420

45 gtt gct tta gcc act gct tca tca ctt gcc gct ggc gtg gtt ttg ggt 1590
 Val Ala Leu Ala Thr Ala Ser Ser Leu Ala Ala Gly Val Val Leu Gly
 425 430 435

50 gag tgg att gcc cgc agg cta cgt cgt cca cca cgc ttc aac cca tac 1638
 Glu Trp Ile Ala Arg Arg Leu Arg Arg Pro Pro Arg Phe Asn Pro Tyr
 440 445 450

55 cgt gca ttt acc aag gcg aat gag ttc tcc ttc cag gag gaa gct gag 1686
 Arg Ala Phe Thr Lys Ala Asn Glu Phe Ser Phe Gln Glu Glu Ala Glu
 455 460 465

60 cag aat cag cgc cgg cag aga aaa cgt cca aag act aat cag aga ttc 1734
 Gln Asn Gln Arg Arg Gln Arg Lys Arg Pro Lys Thr Asn Gln Arg Phe
 470 475 480 485

ggt aat aaa agg taaaaatcaa cctgcttagg cgtctttcgc ttaaatagcg 1786
 Gly Asn Lys Arg

65 tagaatatcg ggtcgatcgc ttttaaacac tcaggaggat ccttgccggc caaaatcacg 1846

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gacactcgtc ccaccccaga atcccttcac gctgttgaag aggaaaccgc agccggggta 1906

ccg

1909

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<210> 4

<211> 489

<212> PRT

<213> Corynebacterium glutamicum ATCC13032

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<400> 4

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35 40 45

20

Asp Ile Leu Leu Ser Ser Gly Thr Ser Asn Ser Asp Thr Lys Val Gln
50 55 60

25

Val Arg Ala Val Thr Ser Ala Tyr Gly Leu Tyr Tyr Thr His Val Asp
65 70 75 80Ile Thr Leu Asn Thr Ile Thr Ile Phe Thr Asn Ile Gly Val Glu Arg
85 90 95

30

Lys Met Pro Val Asn Val Phe His Val Val Gly Lys Leu Asp Thr Asn
100 105 110Phe Ser Lys Leu Ser Glu Val Asp Arg Leu Ile Arg Ser Ile Gln Ala
115 120 125

35

Gly Ala Thr Pro Pro Glu Val Ala Glu Lys Ile Leu Asp Glu Leu Glu
130 135 140

40

Gln Ser Pro Ala Ser Tyr Gly Phe Pro Val Ala Leu Leu Gly Trp Ala
145 150 155 160Met Met Gly Gly Ala Val Ala Val Leu Leu Gly Gly Gly Trp Gln Val
165 170 175

45

Ser Leu Ile Ala Phe Ile Thr Ala Phe Thr Ile Ile Ala Thr Thr Ser
180 185 190Phe Leu Gly Lys Lys Gly Leu Pro Thr Phe Phe Gln Asn Val Val Gly
195 200 205

50

Gly Phe Ile Ala Thr Leu Pro Ala Ser Ile Ala Tyr Ser Leu Ala Leu
210 215 220

55

Gln Phe Gly Leu Glu Ile Lys Pro Ser Gln Ile Ile Ala Ser Gly Ile
225 230 235 240Val Val Leu Leu Ala Gly Leu Thr Leu Val Gln Ser Leu Gln Asp Gly
245 250 255

60

Ile Thr Gly Ala Pro Val Thr Ala Ser Ala Arg Phe Phe Glu Thr Leu
260 265 270Leu Phe Thr Gly Gly Ile Val Ala Gly Val Gly Leu Gly Ile Gln Leu
275 280 285

65

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Ser Glu Ile Leu His Val Met Leu Pro Ala Met Glu Ser Ala Ala Ala
290 295 300

Pro Asn Tyr Ser Ser Thr Phe Ala Arg Ile Ile Ala Gly Gly Val Thr
305 310 315 320

Ala Ala Ala Phe Ala Val Gly Cys Tyr Ala Glu Trp Ser Ser Val Ile
325 330 335

10 Ile Ala Gly Leu Thr Ala Leu Met Gly Ser Ala Phe Tyr Tyr Leu Phe
340 345 350

Val Val Tyr Leu Gly Pro Val Ser Ala Ala Ala Ile Ala Ala Thr Ala
355 360 365

15 Val Gly Phe Thr Gly Gly Leu Leu Ala Arg Arg Phe Leu Ile Pro Pro
370 375 380

20 Leu Ile Val Ala Ile Ala Gly Ile Thr Pro Met Leu Pro Gly Leu Ala
385 390 395 400

Ile Tyr Arg Gly Met Tyr Ala Thr Leu Asn Asp Gln Thr Leu Met Gly
405 410 415

25 Phe Thr Asn Ile Ala Val Ala Leu Ala Thr Ala Ser Ser Leu Ala Ala
420 425 430

Gly Val Val Leu Gly Glu Trp Ile Ala Arg Arg Leu Arg Arg Pro Pro
435 440 445

30 Arg Phe Asn Pro Tyr Arg Ala Phe Thr Lys Ala Asn Glu Phe Ser Phe
450 455 460

35 Gln Glu Glu Ala Glu Gln Asn Gln Arg Arg Gln Arg Lys Arg Pro Lys
465 470 475 480

Thr Asn Gln Arg Phe Gly Asn Lys Arg
485

sub B1
cont.

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